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Laboratory Evaluation of *Custard apple* Seed Extract against Pigeon pea Pod Borer *Helicoverpa armigera* (*Hubner*)

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Abstract
Helicoverpa armigera is the most destructive pest of pigeonpea and high infestation level of this pest causes severe yield losses. The excessive use of synthetic pesticides increase the total cost of operation and also affect the health of human beings adversely. In spite of these, the use of botanicals pesticides are less expensive, safe and eco-friendly. In this study, the aqueous custard apple seed extract with five concentrations (20 mg/ml, 30 mg/ml, 40mg/ml, 50mg/ml and 60mg/ml) were prepared and the efficacy of test solutions were evaluated against *Helicoverpa armigera* larvae under laboratory condition. The lethal concentration LD50 and LC90 of the aqueous custard apple seed extract was found to be 53.24 mg/ml and 107.52 mg/ml, respectively. The results showed that the test solution provides effective control of *Helicoverpa armigera* larval and can be used as botanical insecticide.

Keywords: Custard apple seed, *Helicoverpa armigera* larvae, Mortality, Pesticides, Pigeonpea

1. Introduction
Pigeonpea (*Cajanus cajan* L. Millspaugh) is an important pulse crop which covered near about 5 million ha area globally. In India, it is the second most cultivatable pulse crop after chickpea which cultivated on about 3.62 million ha area having 3.07 million tonnes production [18]. Though, the area under cultivation for pigeonpea crop is increasing every year but the crop yield is still low and remain stagnant. One of the major factors that attribute the low yield of pigeonpea is the damage caused by insect pests. Among them, *Helicoverpa armigera* is the most destructive pest which alone contribute 80-90% of the total damage by the pod borders [11] and the losses due to this pest in pigeonpea is costs more than US $ 300 million annually [7]. Although, the synthetic chemical pesticides significantly contribute in insect pest management, but the excessive and injudicious use of such pesticides results in development of heritable resistance in insects and adversely affect the health of agricultural workers and food consumers [5]. In order to avoid the problems, the natural product-based pesticides can be used as promising alternatives to the chemical pesticides [6, 9]. The bio pesticides are progressively adopted by the farmers due to their eco-friendly nature, low price and safety towards human being. These can greatly reduce the use of synthetic pesticides without affect the yield.

The various plant derived products from different plant families have been traditionally employed as pesticide. Custard apple (*Annona squamosa*) belongs to the Annonaceae plant family. It is a native of the West Indies, it is widely grown in India. Various parts of custard apple plant like leaves and seeds possess the insecticidal properties and these parts were effectively used in various pest management studies [1, 10, 17]. With these views, the present study was undertaken to evaluate the toxicity of custard apple aqueous seed extract against *Helicoverpa armigera* on pigeon pea under laboratory condition.

2. Materials and methods
2.1 Preparation of seed extract
The mature custard apple fruits were collected from local market and the seeds were separated from the fruits manually. The separated seeds were washed with water and shade dried. The dried seeds were crushed using hammer mill and the grinded material was screened through fine mesh sieve. For preparation of 2 percent aqueous seed extract, the 20 g of seed powder was mixed with 1 liter distilled water and this mixture was soaked overnight.

It was then screen through muslin cloth and the volume of the passing liquid (extract) was maintained to 1 liter by adding distilled water. Similarly, 3, 4, 5 & 6 per cent aqueous seed extracts were also prepared and the freshly prepared extracts were used for laboratory evaluation. Distilled water was used as control.

2.2 Bioassay Study

Helicoverpa armigera (*Hubner*) larvae were collected from the infested pigeon pea field. The healthy third-instar larvae were separated and used in bioassay due to their more tolerance against insecticides. The selected larvae were exposed to control and five different concentrations of aqueous custard apple seed extract using leaf dip bioassay method. In this method the unsprayed leaves of pigeon pea were collected from the field, washed and dipped for 10 seconds in aqueous extracts of different concentrations and control. These leaves were shade dried and placed in plastic vials. After that the third-instar larvae were released in each vial and the fed with treated pigeon pea leaves. 30 larvae (3^{rd} instar) per treatment were used and each treatment was replicated thrice. The mortality was recorded after 48 hrs of exposure. The lethal concentrations (LC50 and LC90) and 95% confidence limits of aqueous custard apple seed extracts against *Helicoverpa armigera* were calculated by using the EPA Probit Analysis Program (Version 1.5). The data were accepted if calculated chi square for heterogeneity was lower than the tabular value at the 0.05 level.

3. Results

The aqueous custard apple seed extracts with five different concentrations were prepared and the toxicity test was conducted to evaluate the efficacy of test solution against pigeon pea pod borer *Helicoverpa armigera (Hubner)* larvae under laboratory condition. The mortality of larvae was recorded after 48 hrs of exposure and obtained data were statistically analyzed using EPA Probit Analysis Program (Version 1.5). The mortality rate of *Helicoverpa armigera* (*Hubner*) larvae with different concentrations of aqueous custard apple seed extract and control after 48 hrs exposure is presented in Table 1. The data from Table 1 showed that the larval mortality rate was increased with increase of concentration of custard apple seed extract. The larval mortality with control solution was 10% which may be considered as natural larval mortality. The aqueous custard apple seed extract showed highest mortality (66.67%) against *Helicoverpa armigera* with 6% concentration. The Acute 48 h toxicity of aqueous custard apple seed extract to *Helicoverpa armigera (Hubner)* larvae obtained from the probit analysis is given in Table 2. The value of median lethal toxicity (LD50) and LC90 of the aqueous custard apple seed extract was found to be 53.24 mg/ml and 107.52 mg/ml, respectively. The 95% lower and upper confidence limits for LC50 were 42.84 mg/ml and 72.15 mg/ml, respectively. The approximate 50% population of *Helicoverpa armigera (Hubner)* larvae could be destroyed in the 95% confidence limits. The Plot of adjusted probits and predicted regression line is shown in Fig. 1.

Table 1: The relation between the aqueous custard apple seed extract concentration and the mortality rate of *Helicoverpa armigera* (*Hubner*) larvae.

Concentration (% w/v)	Number exposed	Number responding	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
Control	30	3	0.1000	0.0000	0.1138
2.000	30	5	0.1667	0.0596	0.0371
3.000	30	8	0.2667	0.1725	0.1478
4.000	30	10	0.3333	0.2477	0.3011
5.000	30	15	0.5000	0.4358	0.4544
6.000	30	20	0.6667	0.6238	0.5863

Table 2: Acute 48 h toxicity of aqueous custard apple seed extract to *Helicoverpa armigera* (*Hubner*) larvae.

Point	Exposure concentration (% w/v)	95% confidence limits	Intercept ± SE	Slope ± SE
LC 1.00	1.486	0.115-2.486	1.950963±0.972162	4.198380±1.445853
LC 5.00	2.160	0.358-3.135		
LC 10.00	2.636	0.656-3.562		
LC 15.00	3.016	0.983-3.896		
LC 50.00	5.324	4.284-7.215		
LC 85.00	9.399	7.031-35.482		
LC 90.00	10.752	7.672-53.297		
LC 95.00	13.123	8.700-97.721		
LC 99.00	19.069	10.955-306.300		

Chi-Square for Heterogeneity (calculated) = 0.633; Chi-quare for Heterogeneity (tabular value at 0.05 level) = 7.815

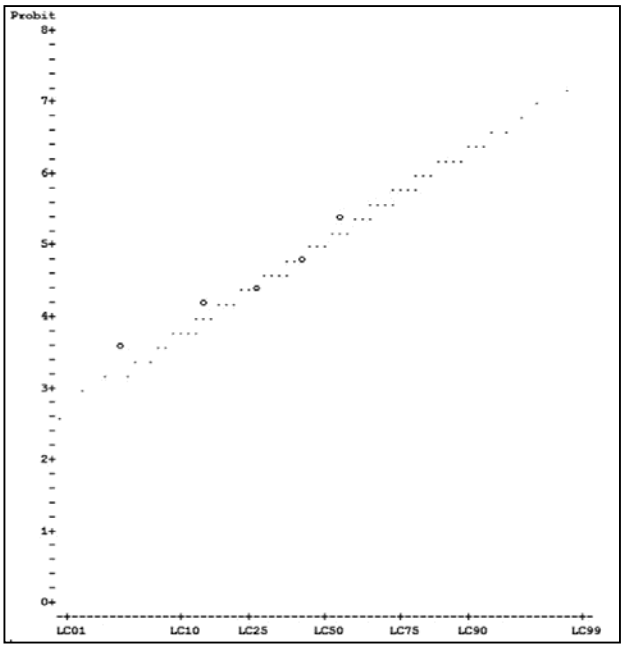

Fig 1: Plot of adjusted probits and predicted regression line.

4. Discussions

In the present study, the efficacy of custard apple aqueous seed extract was tested against *Helicoverpa armigera*. This pest has already developed resistance to most synthetic insecticides [2, 14, 16]. From various studies, it is evident that the botanical insecticides like Neem seed kernel extract, karanj oil, tobacco leaf extract can control *Helicoverpa armigera* effectively [3, 4, 8, 15]. Similar to other botanical insecticides, the custard apple aqueous seed extract was also used in various pest management studies. The aqueous custard apple seed extract was reported for effective control against diamondback moth (*Plutella xylostella*) on cabbage [12] and silverleaf whitefly on tomato plants [13]. In the present study, it was observed that the larval mortalities increased with the concentration of test solution. The custard apple aqueous seed extract at high concentration showed maximum toxicity against *Helicoverpa armigera*. This insecticidal activity of custard apple seeds is due to the chemical compound known as acetogenins which is toxic to insects [17]. The results of this study indicated that the aqueous custard apple seed extract could provide significant larval control of *Helicoverpa armigera* (*Hubner*) by reducing the survival of larvae feeding on pigeon pea leaves treated with the extract.

5. Conclusion

From the results of the present study, it can be concluded that the aqueous custard apple seed extract can be used as botanical insecticide against pod borer *Helicoverpa armigera (Hubner)* in pigeon pea fields. The local availability of custard apple seeds, simple method for preparation of aqueous seed extract, low cost and high efficacy against *Helicoverpa armigera* (*Hubner*) larvae make it suitable option for local use by farmers.

6. References

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